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Do *Ralstonia solanacearum*-Resistant Tomato Varieties Withstand Root-Knot Nematodes? A Greenhouse Evaluation of *Meloidogyne enterolobii* Impact

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Authors' contributions

This work was carried out in collaboration among all authors. Author ENDC wrote the first draft of the research protocol, implemented the experiments, collected the data, performed the statistical analysis, and wrote the first draft of the manuscript. Author AA conceptualized the study, finalized the research protocol, supervised the data collection, revised and edited the manuscript. Author BEH contributed to the implementation of the trial and data collection. Authors SA, CAD and FJ-BQ critically reviewed the protocol and manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Root-knot nematode, *Meloidogyne enterolobii*, is an emerging threat to tomato production due to its wide host range and ability to overcome existing resistance. This study evaluated whether tomato varieties resistant or susceptible to *Ralstonia solanacearum* also exhibit resistance to *M. enterolobii*. Twelve tomato genotypes, AVTO 1955-15, CLN 2498D, CLN 4018G, CLN 4270F, Padma 108 F1, F1 Cobra 26, F1 Thorgal, F1 Mongal, Petomech +, Tropimech, Akikonkouin and Tounvi, were screened under greenhouse conditions by inoculating each plant with 1,000 second-stage juveniles (J2) and eggs of *M. enterolobii* in the first experiment and 1,500 nematodes in the repeat experiment. Both experiments were laid out in completely randomized design with five replicates. Nematode reproduction factor (RF), galling index (GI), plant growth parameters, and physiological traits such as chlorophyll content and photochemical yield (Fv/Fm) were assessed. At harvest, the GI values ranged from 4.0 to 7.8 and RF values > 2. This suggests that all varieties were susceptible to *M. enterolobii* under tested conditions, although F1 Cobra 26 and CLN 4018G showed relatively lower nematode reproduction and root galling. Nematode infection significantly reduced plant growth and photosynthetic efficiency. These findings highlight the need for integrated nematode management and support the inclusion of nematode resistance screening in tomato breeding programs, particularly for dual-pathogen environments.

Keywords: Bacterial wilt; root-knot nematodes; screening for resistance; Solanum lycopersicum; vegetable crops.

1. INTRODUCTION

Plant-parasitic nematodes (PPNs) are microscopic worms that feed on plant tissues, causing severe crop yield losses estimated at 14%, corresponding to approximately $125 billion annually worldwide (Abd-Elgawad and Askary, 2015; Mesa-Valle et al., 2020). Among these, root-knot nematodes (*Meloidogyne* spp., RKNs) represent the most economically damaging group (Jones et al., 2013; Bhat et al., 2023) and are a key constraint to tomato production, particularly in tropical regions (Coyne et al., 2018; Hallmann and Meressa, 2018). RKNs disrupt water and nutrient uptake by inducing gall formation on roots, leading to impaired plant physiology (Walia and Khan, 2023). Infested tomato plants exhibit yellowing leaves, premature leaf drop, and reduced growth and development (Desaeger et al., 2023). Furthermore, RKNs predispose infested crops to secondary infections by fungi, bacteria, and viruses, leading to increased yield losses (Walia and Khan, 2023).

The soil-borne bacterium *Ralstonia solanacearum*, one of the most devastating pathogens of vegetable crops (Wang et al., 2025), has been reported to interact with RKNs. In tropical and subtropical regions where *R. solanacearum* is primarily found, the bacterial wilt caused by this pathogen can severely reduce the yield and quality of vegetable crops (Zhao et al., 2023; Parrado and Quintanilla, 2024). Under favourable conditions, *R. solanacearum* can cause 72-100% yield losses in tomato (Sikirou et al., 2017; Dossoumou et al., 2023). Previous reports also indicated losses of 50-100% and up to 100% in other crops, such as potato, ginger, banana, tobacco, and peppers (Habetewold et al., 2015; Mamphogoro et al., 2020; Ahmed et al., 2022).

Host plant resistance has been considered as a suitable approach to control *R. solanacearum* and a number of resistant varieties have been developed (Oussou et al., 2020; Zohoungbogbo et al., 2021). However, Deberdt et al. (1999) indicated that RKN infestation compromises bacterial wilt resistance in tomatoes through physical disruption of roots tissues induced by gall formation, thereby facilitating *R. solanacearum* invasion in resistant varieties. This synergistic interaction has been widely documented across solanaceous crops. Previous studies on pepper have confirmed that co-infection with *R. solanacearum* and RKN exacerbates the severity of bacterial wilt (Asghar et al., 2020; Wang et al., 2025). RKN infection creates entry points that enable vascular

colonization by *R. solanacearum* (Kidane et al., 2019; Junaid et al., 2020), with nematode galls providing ideal micro-environments for bacterial proliferation (Wang et al., 2025). Such dual-pathogen synergism represents a threat to crop production.

Considering the wide geographical distribution of RKNs and *R. solanacearum* and their co-existence, particularly in vegetable fields (Furusawa et al., 2019; Archana et al., 2023; Azandeme-Hounmalon et al., 2023), it is essential to provide growers with varieties resistant to both pathogens or appropriate crop protection options. Therefore, this study evaluated whether commercial hybrids and newly introduced tomato varieties, including those resistant to *R. solanacearum,* exhibit resistance to *Meloidogyne enterolobii*.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted under greenhouse conditions at the Nematology Unit (UNema) research station in Sekou, Benin (06°37'30.8" N; 002°13'56.9" E; 74 m asl) from November 2023 to June 2024. The research station is located in the Guinean biogeographical zone and characterized by a sub-equatorial climate with two wet seasons (April to mid-July and mid-September to end October) and two dry seasons (Affokpon et al., 2021).

2.2 Planting Material

Twelve tomato varieties with different resistance levels to *Ralstonia solanacearum* (Table 1) were assessed for their resistance to *Meloidogyne enterolobii* infestation. They included six commercial hybrids cultivated in West Africa, four varieties developed by the World Vegetable Center (WorldVeg), and two local cultivars from Benin used as controls.

2.3 Preparation of Nematode Inoculum

A pure population of *M. enterolobii* maintained in the greenhouse of the Nematology Unit was multiplied in 3-liter pots on tomato *cv. Tounvi* for 90 days. Eggs and second-stage juveniles (J_2) were extracted from infested roots using the sodium hypochlorite (NaOCl) method (Coyne and Ross, 2014). Briefly, infested roots were washed, cut into small segments, and blended in 0.5% NaOCl solution. The resulting suspension was filtered through a series of sieves (200, 100, 38, and 25 µm), and the eggs and J_2 retained on the 25 µm-sieve were collected. Subsequently, the inoculum density was quantified based on five nematode counts under an optical microscope at 40x magnification.

2.4 Experimental Details and Design

The experiment was conducted in November 2023 and repeated once in April 2024. The pots were arranged in a completely randomized design with five replicates. Three-week-old seedlings grown in sterilized soil were individually transplanted into 1.5-L pots filled with 1000 cm^3 of sterilized substrate (1:1 sand-topsoil mixture, loam-sandy texture: 76.56% sand, 16.2% silt, 6.4% clay; organic matter: 1.90%; C/N ratio: 16.97; EC: 0.1 dSm^{-1} and pH (H_20): 6.47). Seven days after transplanting, a freshly prepared, counting dish-standardized suspension of 1000 *M. enterolobii* (24% hatched J_2 and 76% eggs) in 1.5 ml was inoculated into four 2 cm-deep root zone holes in each pot. In the repeat experiment, seedlings were transplanted into 2-L pots filled with 1500 cm^3 of the same substrate and individually inoculated with 1.5 ml suspension containing 1500 nematodes (7% J_2 and 93% eggs). Plants were watered twice a day with 25–50 ml tap water and fertilized with 2% NPK (15-15-15) 35 days after transplanting. Daily temperatures and relative humidity (RH) were recorded throughout the experiments using digital thermo-hygrometer placed inside the greenhouse. During the first experiment, the temperatures ranged from 27°C to 39°C and RH was between 44% and 95%. During the repeat experiment, the temperatures varied from 27°C to 38°C and the RH was between 66% and 95%. Each experiment was concluded eight weeks after nematode inoculation.

2.5 Assessment of Nematode Population Densities and Root Galling

In both experiments, nematode population densities and root galling were assessed at harvest. Plants were uprooted, and the root system was used to assess the galling index (GI) and the numbers of galls, egg masses, and eggs per egg mass. GI was estimated on the entire root system using 0-10 scale, where 0 = no galling damage and 10 = 91-100 % galled roots (Affokpon et al., 2012). Visible galls were counted per gram of roots. The number of egg masses was determined after the roots were stained with 20% McCormick red food colour for

Table 1. Bacterial wilt host status of the tomato varieties

Tomato varieties	Type of varieties	Bacterial wilt status	Reference/Source
AVTO 1955-15	WorldVeg variety	Resistant	Zohoungbogbo et al., 2021
CLN 2498D	WorldVeg variety	Resistant	WorldVeg Online Catalog
CLN 4018G	WorldVeg variety	Resistant	WorldVeg Online Catalog
CLN 4270F	WorldVeg variety	Resistant	WorldVeg
Padma 108 F1	Commercial hybrid	Highly resistant	Dossoumou et al., 2021
F1 Cobra 26	Commercial hybrid	Moderately resistant	Dossoumou et al., 2021
F1 Thorgal	Commercial hybrid	Moderately resistant	Oussou et al., 2020
F1 Mongal	Commercial hybrid	Moderately resistant	Oussou et al., 2020
Petomech +	Commercial hybrid	Susceptible	Provider sheet ; Dossoumou et al., 2021
Tropimech	Commercial hybrid	Highly susceptible	Dossoumou et al., 2021
Akikonkouin	Local	Highly susceptible	Dossoumou et al., 2021
Tounvi	Local	Highly susceptible	Dossoumou et al., 2021

15 minutes. Ten egg masses were then randomly removed from each root system and crushed in 10-ml sterile distilled water using slides to release eggs. Thereafter, eggs were counted from 2 x 1 ml aliquots under an optical microscope (Euromex iScope IS.1153-PLi) at 40x magnification to estimate the average number of eggs per egg mass for each plant.

Thereafter, final nematode population densities were determined per pot by extracting J_2 and eggs from 250 cm^3 soil and 5 g root sub-samples using the centrifugation technique described by Affokpon et al. (2011). The reproductive factor (RF) was then calculated as the ratio between the total number of soil and root J_2 and eggs per pot at harvest and the initial nematode inoculum.

Based on GI and RF, tomato varieties were categorized into four groups: resistant (GI ≤ 2 and RF ≤ 1), tolerant (GI ≤ 2 and RF > 1), hypersusceptible (GI > 2 and RF ≤ 1), and susceptible (GI > 2 and RF > 1) (Sasser et al., 1984).

2.6 Measurement of Crop Growth Characters

At the end of each experiment, the shoot length, root length, stem girth, number of leaves, shoot fresh weight, root fresh weight, and shoot dry weight were determined. Shoot and root lengths were measured using a centimeter scale and stem girth with a digital Vernier caliper. Shoot and root fresh weight was measured immediately after uprooting the plants using a precision electronic balance, with roots being carefully washed and blotted dry. Shoot dry weight was obtained after oven drying at 60°C for 72 hours.

2.7 Measurement of Physiological Parameters

Physiological parameters, such as leaf photochemical yield (Fv/Fm) and chlorophyll content, were additionally assessed in the repeat experiment. Three well-developed leaves were selected from the upper portion of each plant and used to evaluate each parameter. Fv/Fm was then measured using a portable fluorometer (Model OS-30P+), and chlorophyll content was quantified with a Soil Plant Analysis Development (SPAD)-502 Plus chlorophyll meter. Values from the three leaves were averaged to generate a single data point per plant.

2.8 Statistical Analyses

Statistical analyses were performed using R software version 4.4.2 (R Core Team, 2024). Each experiment was analyzed separately because the variety x experiment interaction was significant at 5%. Prior to statistical analysis, counting data were $\log_{10}(x+1)$-transformed to conform to normal distribution (Gomez and Gomez, 1984). Within each experiment, all data were subjected to one-way analysis of variance (ANOVA), and means were separated by Tukey's Honest Significant Difference (HSD) test at a 5% level of significance. To understand the relationships between key nematological, plant growth, and physiological parameters, Pearson's correlation coefficient was computed in the repeat experiment and correlation matrix was visualized using the "ggcorrplot" R-package (Kassambara, 2019).

3. RESULTS AND DISCUSSION

3.1 Nematode Population Densities and Damage

In the first experiment, final nematode population densities, RF, and GI varied significantly (*P < .001*) between tomato varieties (Table 2 and Supplementary Table 1). The variety CLN 4018G had the lowest juvenile densities in roots, with a reduction of 58% compared with the control Tounvi (Supplementary Table 1). Although inter-varietal differences in egg numbers were observed among the improved varieties, they did not outperform the local cultivars overall (Supplementary Table 1). Five varieties showed significantly lower RF than the two local cultivars, among which the commercial hybrids F1 Cobra 26 and F1 Mongal and the WorldVeg variety CLN 4018G had RF ≤ 2.75 (Table 2).

Table 2. Nematode reproductive factor (RF) and gall index (GI) on tomato varieties eight weeks after inoculation with 1000 *M. enterolobii* in 1000 cm³ sterilized soil

Varieties	*RF = Pf/Pi*[a]	Gall index[b] (0-10)
F1 Cobra 26	2.46 f	5.4 fg
CLN 4018G	2.54 f	7.0 bc
F1 Mongal	2.75 f	5.2 g
F1 Thorgal	4.01 ef	7.4 ab
CLN 2498D	4.02 def	6.0 ef
CLN 4270F	4.59 cde	7.8 a
AVTO 1955-15	5.25 cde	6.8 bcd
Padma 108 F1	5.76 cd	6.4 cde
Akikonkouin	5.88 c	5.4 fg
Tropimech	5.95 c	5.2 g
Petomech +	7.74 b	7.2 ab
Tounvi	10.72 a	6.2 de
Fisher	*43.6*	*11.34*
P value	*< .001*	*< .001*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]RF (nematode reproductive factor) = final density of eggs and juveniles per pot / initial nematode inoculum per pot. [b]Gall index was assessed on a scale of 0-10, where 0 = no galling and 10 = 91-100 % galled roots (Affokpon et al*., 2012)*

Table 3. Nematode reproductive factor (RF) and gall index (GI) on tomato varieties eight weeks after inoculation with 1500 *M. enterolobii* in 1500 cm³ sterilized soil

Varieties	*RF = Pf/Pi*[a]	Gall index[b] (0-10)
F1 Cobra 26	2.01 c	5.8 abc
CLN 4018G	2.30 c	6.6 ab
CLN 4270F	2.45 c	6.4 ab
AVTO 1955-15	3.73 c	6.6 ab
F1 Thorgal	3.79 c	4.4 cd
Akikonkouin	3.79 c	5.6 abcd
CLN 2498D	4.64 c	6.8 a
Padma 108 F1	5.79 c	4.0 d
Tropimech	6.01 bc	5.0 bcd
F1 Mongal	10.10 ab	6.8 a
Petomech +	12.28 a	5.8 abc
Tounvi	13.34 a	5.6 abcd
Fisher	*20.440*	*2.47*
P value	*< .001*	*.015*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]RF (nematode reproductive factor) = final density of eggs and juveniles per pot / initial nematode inoculum per pot. [b]Gall index was assessed on a scale of 0-10, where 0 = no galling and 10 = 91-100 % galled roots (Affokpon et al*., 2012)*

The galling index varied between 5.2 and 7.8 over 10, ranging from moderate to high damage levels (Table 2). The commercial varieties F1 Mongal, Tropimech, and F1 Cobra 26 showed the lowest GI (≤ 5.4), which was significantly lower than that of the local "Tounvi" (6.2). Similar trends were observed for egg mass production, with F1 Cobra 26 and Tropimech having significantly fewer number of egg masses per g roots than the local cultivar "Akinkonkouin" (Supplementary Table 2). Among the ten varieties assessed in this study, seven had significantly lower numbers of eggs per egg mass than the local cultivars (Supplementary Table 2).

In the repeat experiment, nematode multiplication and root galling varied significantly (*P* < *.001*) between varieties (Table 3). Compared with the local cultivars "Akikonkouin" and "Tounvi", the juvenile population densities in the roots of the variety F1 Cobra 26 were significantly lower (reduction by up to 87%) (Supplementary Table 3). The nematode RF was significantly lower in most varieties than in the local cultivar "Tounvi", but remained similar to that in the local "Akikonkouin" (Table 3). Varieties F1 Cobra 26, CLN 4018G, and CLN 4270F exhibited the lowest RF values (2.0 - 2.5).

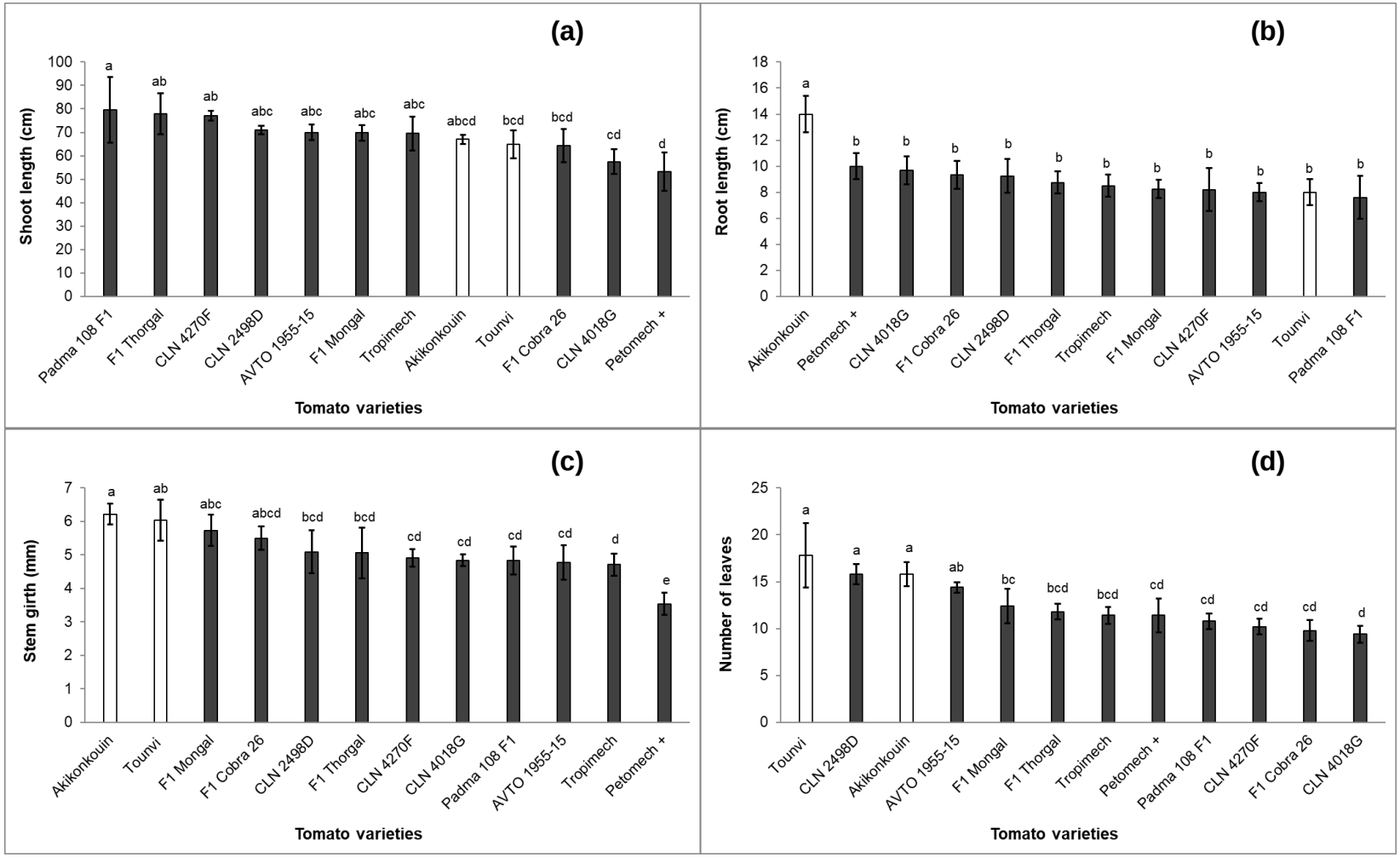
The galling index varied between 4 and 6.8 (Table 3). Although they differed significantly among improved varieties, no significant reduction was observed between improved varieties and local cultivars. Similar observations were reported for the mean number of galls (Supplementary Table 4). F1 Mongal had the lowest number of egg masses, with 54% and 75% reductions compared with the local cultivars "Tounvi" and "Akinkonkouin", respectively. The number of eggs per egg mass varied significantly between improved varieties, but was similar to that of the local cultivar (Supplementary Table 4).

Our study showed significant differences in final nematode populations and root galling among tomato varieties, reflecting varying degrees of responses to *M. enterolobii* infection and variability in genetic background. In previous studies, many authors have reported variability in the responses of tomato varieties to RKN infections in various screening pot experiments (Seid et al., 2017; Dhillon et al., 2020; Markova et al., 2024). All the varieties assessed against *M. enterolobii* had GI ≥ 4 and RF > 2, corresponding to susceptible cultivars according to the scale established by Sasser et al. (1984). This result underscores the ability of *M. enterolobii* to reproduce in all these varieties. *Meloidogyne enterolobii* is known to be aggressive and capable of overcoming traditional plant resistance mechanisms (Collett et al., 2021). Karssen and Moens (2006) and Dhillon et al. (2020) noted that susceptible genotypes allowed nematodes to penetrate and develop in roots, leading to high reproduction rates, as observed in the local susceptible variety "Tounvi". However, in our study, F1 Cobra 26 and CLN 4018G had consistently lower RF than the local susceptible cultivars despite the difference in initial nematode densities in the two experiments. The findings suggest partial suppression of nematode reproduction, potentially via reduction of the number of eggs per egg mass or inhibition of gall development induced by invading juveniles that could fail to reproduce (Affokpon et al., 2011). The mechanisms underlying this lower susceptibility could be enhanced when these varieties are supplemented with integrated nematode management strategies.

3.2 Effect of Nematode Infections on Plant Growth

The shoot and root length, stem girth, number of leaves, fresh and dry shoot weights, and fresh root weight varied significantly among tomato varieties (*P* < *.001*) (Fig. 1 and Fig. 2). In the first experiment, regardless of the plant growth parameters, none of the commercial or WorldVeg varieties performed better than the local cultivars (Fig.1), except Padma 108 F1, which performed better than Tounvi for shoot length (Fig. 1a). Similar results were observed for the repeat experiment (Fig. 2), except that CLN 4270F outperformed better than Akikonkouin in terms of shoot length (Fig. 2a).

This finding suggests that root galling induced by RKN severely undermines plant growth. Heavy galled roots loose most of the secondary roots, which seriously disturbs the ability of the plants to uptake water and nutrients, resulting in reduction of shoot growth, wilting, and/or yellowing (Hallmann and Meressa, 2018; Desaeger et al., 2023). *Meloidogyne enterolobii* is a global threat to tomato production due to the lack of known resistance in commercially accepted varieties, including those carrying the Mi-1 gene (Seid et al., 2015; Philbrick et al., 2020; Sikandar et al., 2024). Rawat and Kumar (2020) also reported that an inoculum starting from 500 *M. enterolobii* J_2 per kg soil significantly reduced the growth parameters on tomato cv *Bhagya* and *Kashi Anupam*.


(a)
Shoot length (cm)
Padma 108 F1
F1 Thorgal
CLN 4270F
CLN 2498D
AVTO 1955-15
F1 Mongal
Tropimech
Akikonkouin
Tounvi
F1 Cobra 26
CLN 4018G
Petomech +
Tomato varieties
(b)
Root length (cm)
Akikonkouin
Petomech +
CLN 4018G
F1 Cobra 26
CLN 2498D
F1 Thorgal
Tropimech
F1 Mongal
CLN 4270F
AVTO 1955-15
Tounvi
Padma 108 F1
Tomato varieties
(c)
Stem girth (mm)
Akikonkouin
Tounvi
F1 Mongal
F1 Cobra 26
CLN 2498D
F1 Thorgal
CLN 4270F
CLN 4018G
Padma 108 F1
AVTO 1955-15
Tropimech
Petomech +
Tomato varieties
(d)
Number of leaves
Tounvi
CLN 2498D
Akikonkouin
AVTO 1955-15
F1 Mongal
F1 Thorgal
Tropimech
Petomech +
Padma 108 F1
CLN 4270F
F1 Cobra 26
CLN 4018G
Tomato varieties

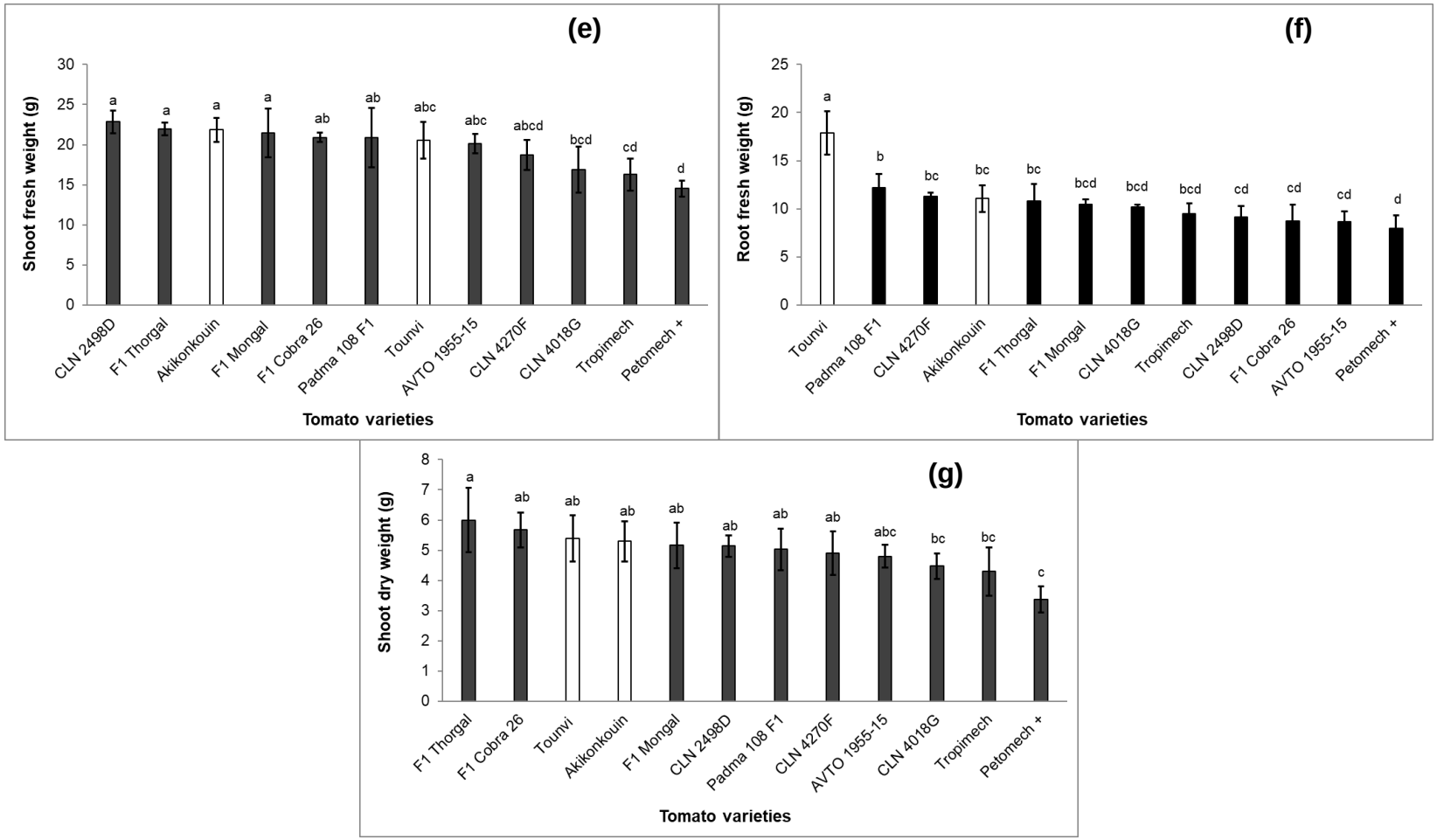
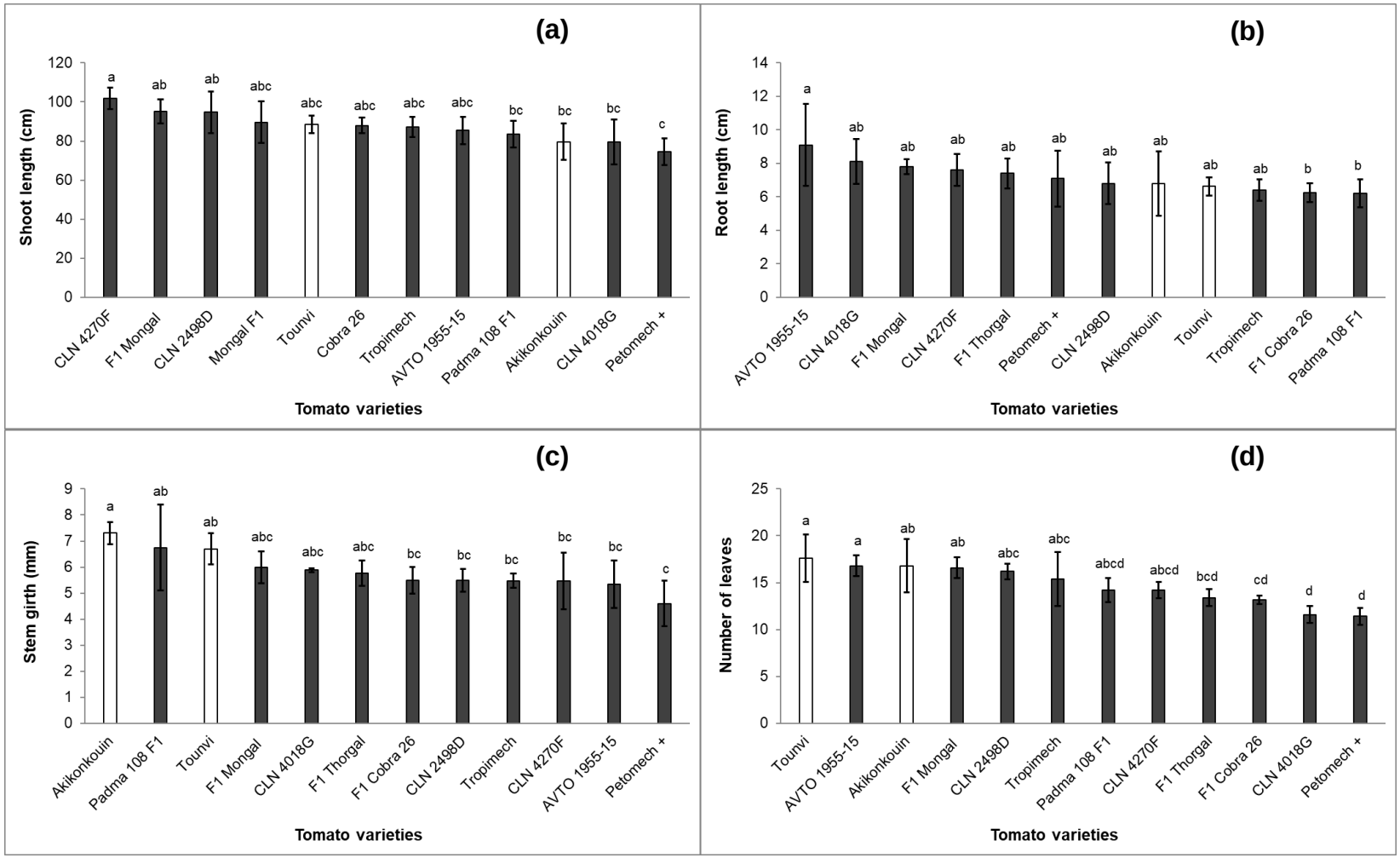


Fig. 1. Shoot length (a), root length (b), stem girth (c), number of leaves (d), shoot fresh weight (e), root fresh weight (f), shoot dry weight (g) of tomato varieties eight weeks after inoculation with 1000 *M. enterolobii* in 1000 cm^3 sterilized soil

Data are means of five replicates. Bars followed by different letters are significantly different ($P \leq .05$) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA


(a)
Shoot length (cm)
120
100
80
60
40
20
0
a
ab
ab
abc
abc
abc
abc
abc
abc
bc
bc
bc
c
CLN 4270F
F1 Mongal
CLN 2498D
Mongal F1
Tounvi
Cobra 26
Tropimech
AVTO 1955-15
Padma 108 F1
Akikonkouin
CLN 4018G
Petomech +
Tomato varieties
(b)
Root length (cm)
14
12
10
8
6
4
2
0
a
ab
ab
ab
ab
ab
ab
ab
ab
ab
b
b
AVTO 1955-15
CLN 4018G
F1 Mongal
CLN 4270F
F1 Thorgal
Petomech +
CLN 2498D
Akikonkouin
Tounvi
Tropimech
F1 Cobra 26
Padma 108 F1
Tomato varieties
(c)
Stem girth (mm)
9
8
7
6
5
4
3
2
1
0
a
ab
ab
abc
abc
abc
bc
bc
bc
bc
bc
c
Akikonkouin
Padma 108 F1
Tounvi
F1 Mongal
CLN 4018G
F1 Thorgal
F1 Cobra 26
CLN 2498D
Tropimech
CLN 4270F
AVTO 1955-15
Petomech +
Tomato varieties
(d)
Number of leaves
25
20
15
10
5
0
a
a
ab
ab
abc
abc
abcd
abcd
bcd
cd
d
d
Tounvi
AVTO 1955-15
Akikonkouin
F1 Mongal
CLN 2498D
Tropimech
Padma 108 F1
CLN 4270F
F1 Thorgal
F1 Cobra 26
CLN 4018G
Petomech +
Tomato varieties

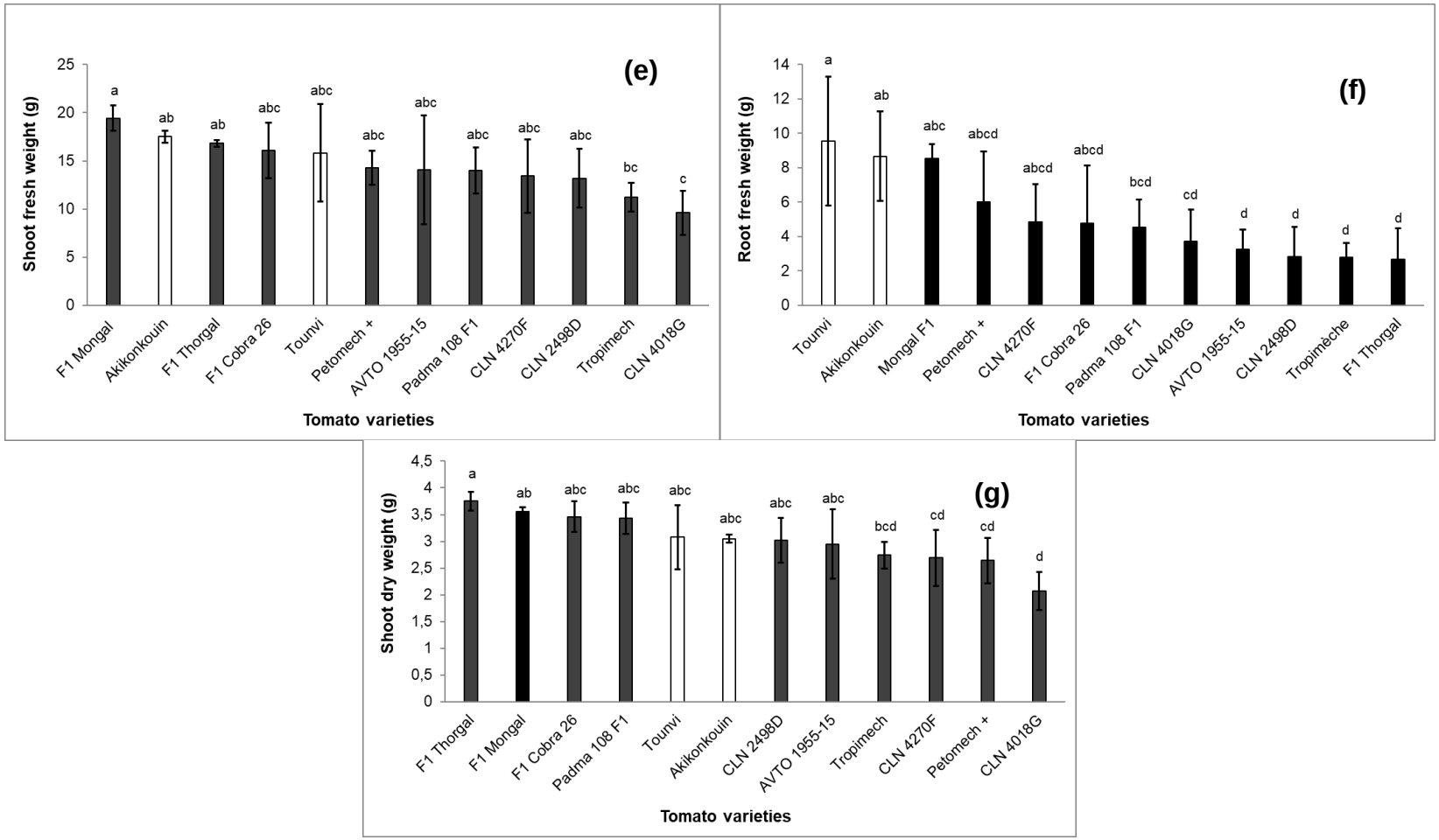


Fig. 2. Shoot length (a), root length (b), stem girth (c), number of leaves (d), shoot fresh weight (e), root fresh weight (f), shoot dry weight (g) of tomato varieties eight weeks after inoculation with 1500 *M. enterolobii* in 1500 cm^3 sterilized soil

Data are means of five replicates. Bars followed by different letters are significantly different ($P \leq .05$) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA

3.3 Physiological traits across varieties and their correlation with nematode infections and tomato growth

Results from the repeat experiment showed significant variations in Fv/Fm (Fig. 3) and leaf chlorophyll content (Fig. 4) across tomato varieties (*P<.001*). Regarding the photochemical traits, very few varieties had higher Fv/Fm than the local cultivar "Tounvi" whereas no significant difference was observed when considering the local cultivar "Akikonkouin" (Fig. 3). Similar results were observed with the leaf chlorophyll content (Fig. 4).

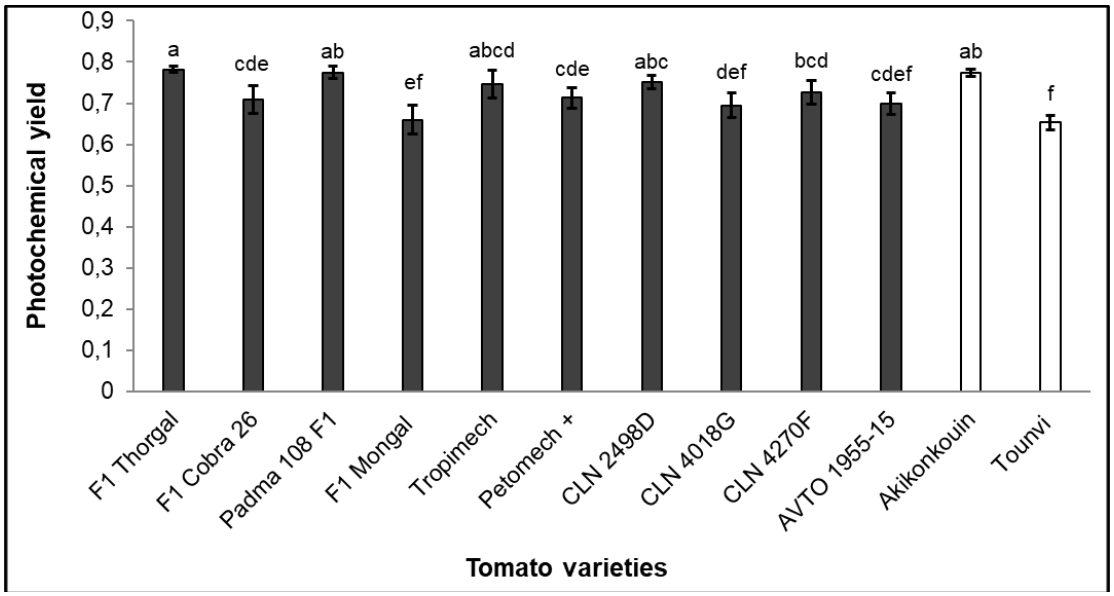


Fig. 3. Photochemical yield (Fv/Fm) of tomato varieties eight weeks after inoculation with 1500 *M. enterolobii*

Data are means of five replicates. Bars followed by different letters are significantly different (P ≤.05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA

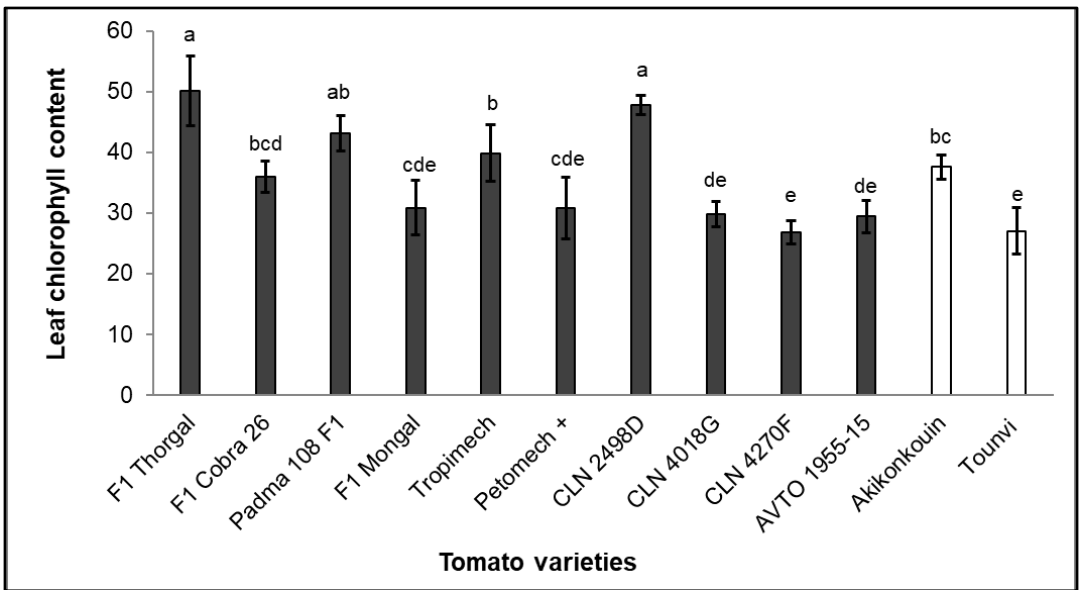


Fig. 4. Leaf chlorophyll content of tomato varieties eight weeks after inoculation with 1500 *M. enterolobii*

Data are means of five replicates. Bars followed by different letters are significantly different (P ≤.05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA

Fv/Fm is an important indicator of plant health, with optimal values of approximately 0.83 in non-stressed conditions (Maxwell and Johnson, 2000) whereas leaf chlorophyll content indicates the photosynthetic capacity of the plant (Jiang et al., 2017). In our study, the observed Fv/Fm (0.65 - 0.78) and chlorophyll content (26,82 - 47,74) suggest that *M. enterolobii* may induce physiological stress in plants following infections, affecting particularly the photosystem II (Ghasemzadeh et al., 2019). Sikandar et al. (2024) also observed a significant decrease in chlorophyll fluorescence parameters and chlorophyll content on tomato plants *cv Zhongza 09* with *M. enterolobii* population density ranging from 100 to 2000 J_2 per plant. According to these authors, chlorophyll content reductions can be attributed to increasing oxidative stress and degradation of chlorophyll pigments due to *M. enterolobii* pressure.

Pearson correlation analysis showed significant associations ($P < .05$) between key nematological, growth, and physiological parameters (Fig. 5). Nematode reproductive factor was negatively correlated with the Fv/Fm (r = -0.41; $P = .0013$). Similarly, the gall index was negatively correlated with the Fv/Fm (r = -0.30; $P = .02$) and leaf chlorophyll content (r = -0.32; $P = .013$). Likewise, root fresh weight was negatively correlated with the Fv/Fm (r = -0.34; $P = .0087$) and chlorophyll content (r = -0.35; $P = .0058$).

The significant correlations between key nematological, growth, and physiological parameters provide valuable information on the host plant-nematode interactions. High reproductive factor and gall index reduced photosynthetic function and chlorophyll levels, suggesting that RKNs may take over plant nutrients to the detriment of plant growth, thus affecting physiological processes such as photosynthesis (Ghasemzadeh et al., 2019; Sikandar et al., 2024). This situation was observed in the repeat experiment with a significant negative correlation between nematode parameters and Fv/Fm and chlorophyll content, resulting in their lower values and reflecting the stress induced by nematode infections.

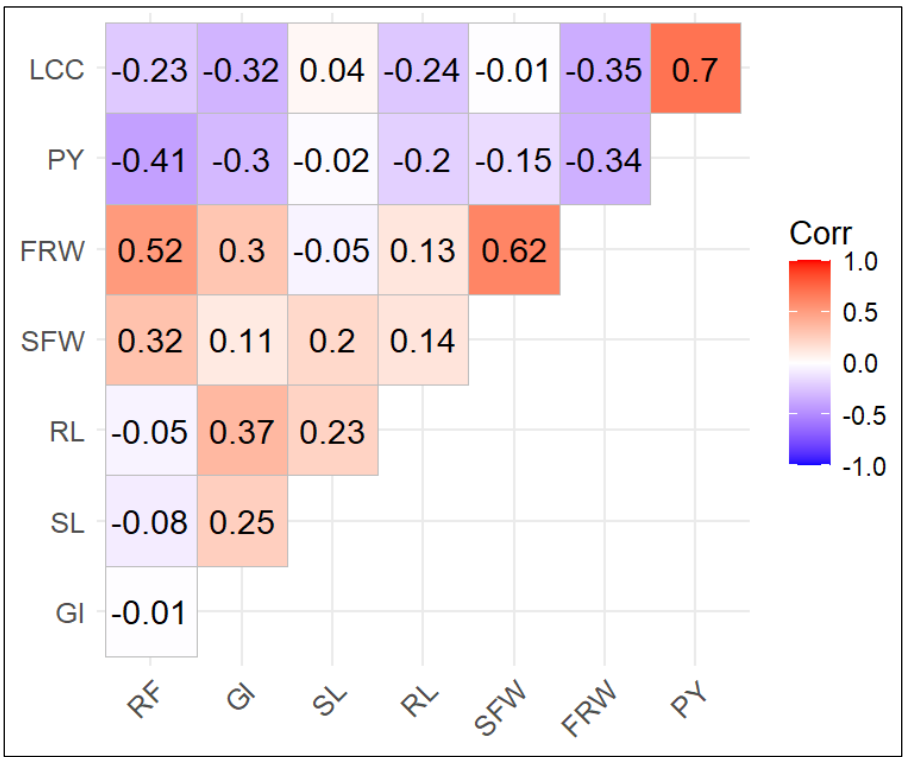


Fig. 5. Pearson's correlation coefficient based on nematological, growth and physiological parameters of tomato varieties eight weeks after inoculation with 1500 *M. enterolobii* in 1500 cm^3 sterilized soil

Pearson coefficients (Corr) are calculated across pooled data from all varieties in the repeat experiment; RF: Reproductive factor; GI: Gall index; SL: Shoot length; RL: Root length; SFW: Shoot fresh weight; FRW: Fresh root weight; PY: Photochemical yield; LCC: Leaf chlorophyll content

4. CONCLUSION

The current study demonstrates that all twelve tomato varieties are susceptible to *M. enterolobii*, thus confirms that developing tomato genotypes resistant to *M. enterolobii* for vegetable growers remains a key challenge. The results point out significant correlations between nematode parameters, plant growth, and physiological traits, showing in particular that the nematode reproductive factor and the galling index were negatively correlated with Fv/Fm and chlorophyll content. Given that RKN infections can weaken the genetic resistance of tomato varieties to *R. solanacearum*, appropriate RKN management strategies should be developed and advised to producers when using these tomato varieties in nematode-infested fields. The valuable information provided in this study calls the attention of breeders to consider nematode-related parameters in the breeding programme against *R. solanacearum*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests.

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Supplementary Table 1. Final root-knot nematode population densities on tomato varieties eight weeks after inoculation with 1000 *M. enterolobii* in 1000 cm³ sterilized soil

Varieties	Juveniles/ 5 g roots[a]	Eggs / 5 g roots[a]	Juveniles/ 250 cm³ soil[a]
F1 Thorgal	4375 bc	192 bc	78 c
F1 Cobra 26	3843 bc	160 c	45 de
Padma 108 F1	5391 b	1072 abc	159 a
F1 Mongal	4258 bc	179 bc	37 ef
Tropimech	6515 b	909 ab	177 a
Petomech +	15168 a	292 abc	25 f
CLN 2498D	3829 bcd	1537 a	76 c
CLN 4018G	2594 c	192 bc	69 cd
CLN 4270F	3922 bc	317 abc	147 ab
AVTO 1955-15	6783 b	403 abc	89 c
Akikonkouin	6149 b	398 abc	180 a
Tounvi	6201 b	687 abc	91 bc
Fisher	*10.91*	*3.754*	*42.65*
P value	*< .001*	*< .001*	*< .001*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]Statistical analysis based on log_{10} (x+1), backtransformed data presented.

Supplementary Table 2. Root galling and egg mass production in tomato varieties eight weeks after inoculation with 1000 *M. enterolobii* in 1000 cm³ sterilized soil

Varieties	no galls/ g of roots[a]	no egg masses/ g of roots[a]	no eggs/ egg mass[a]
F1 Thorgal	362 ab	55 bc	226 ab
F1 Cobra 26	204 e	22 f	155 cd
Padma 108 F1	277 cde	71 ab	267 a
F1 Mongal	236 cde	42 cd	120 d
Tropimech	226 de	26 ef	169 bc
Petomech +	363 ab	54 bc	137 cd
CLN 2498D	267 cde	65 ab	111 d
CLN 4018G	286 cd	32 de	123 d
CLN 4270F	392 a	58 bc	191 abc
AVTO 1955-15	304 bc	89 a	144 cd
Akikonkouin	220 de	35 d	258 a
Tounvi	279 cd	53 bc	260 a
Fisher	*13.1*	*44.32*	*20.7*
P value	*< .001*	*< .001*	*< .001*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]Statistical analysis based on log_{10}(x+1), backtransformed data presented.

Supplementary Table 3. Final root-knot nematode population densities on tomato varieties eight weeks after inoculation with 1500 *M. enterolobii* in 1500 cm³ sterilized soil

Varieties	Juveniles/ 5 g roots[a]	Eggs/ 5 g roots[a]	Juveniles/ 250 cm³ soil[a]	Eggs/ 250 cm³ soil[a]
F1 Thorgal	5761 def	78 bcd	848 ab	2 ab
F1 Cobra 26	1926 i	217 d	354 abc	2 ab
Padma 108 F1	7949 cd	1163 ab	1122 ab	6 ab
F1 Mongal	21346 a	1827 ab	278 bc	3 ab

Varieties	Juveniles/ 5 g roots[a]	Eggs/ 5 g roots[a]	Juveniles/ 250 cm³ soil[a]	Eggs/ 250 cm³ soil[a]
Tropimech	3859 fgh	1014 ab	1311 a	11 ab
Petomech +	21256 a	5275 a	360 abc	0 b
CLN 2498D	6537 cde	174 abcd	1002 ab	43 a
CLN 4018G	2341 hi	92 cd	482 abc	8 ab
CLN 4270F	4636 efg	33 d	390 abc	2 ab
AVTO 1955-15	9411 bc	592 abc	454 abc	2 ab
Akikonkouin	3418 gh	227 bcd	555 abc	6 ab
Tounvi	14862 ab	2621 a	235 c	6 ab
Fisher	*59.34*	*8.652*	*4.001*	*2.048*
P value	*< .001*	*< .001*	*< .001*	*.044*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]Statistical analysis based on $log_{10}(x+1)$, backtransformed data presented.

Supplementary Table 4. Root galling and egg mass production in tomato varieties eight weeks after inoculation with 1500 *M. enterolobii* in 1500 cm³ sterilized soil

Varieties	no galls/ g of roots[a]	no egg masses/ g of roots[a]	no eggs/ egg mass[a]
F1 Thorgal	435 a	181 a	141 ab
F1 Cobra 26	199 ab	88 ab	82 bc
Padma 108 F1	201 ab	80 ab	88 bc
F1 Mongal	133 b	17 c	163 a
Tropimech	262 ab	156 ab	103 abc
Petomech +	202 ab	84 ab	103 abc
CLN 2498D	222 ab	67 ab	78 c
CLN 4018G	289 ab	46 bc	99 abc
CLN 4270F	243 ab	48 bc	144 abc
AVTO 1955-15	185 b	77 ab	146 ab
Akikonkouin	156 b	73 ab	120 abc
Tounvi	197 ab	37 bc	100 abc
Fisher	*3.007*	*5.464*	*3.966*
P value	*.004*	*< .001*	*< .001*

Data are means of five replicates. Values in columns followed by different letters are significantly different (P ≤ .05) based on Tukey's Honest Significant Difference (HSD) test using one-way ANOVA. [a]Statistical analysis based on $log_{10}(x+1)$, backtransformed data presented.

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